BRITISH STANDARD 838 : 1961

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METHODS OF TEST FOR TOXICITY OF WOOD PRESERVATIVES TO FUNGI

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BRITISH STANDARDS INSTITUTION

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METHODS OF TEST FOR
TOXICITY OF
WOOD PRESERVATIVES
TO FUNGI

B.S. 838 : 1961

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THIS BRITISH STANDARD, having been approved by the Wood Preservation Industry Standards Committee and endorsed by the Chairman of the Chemical Divisional Council, was published under the authority of the General Council on 30th March, 1961.

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The Institution desires to call attention to the fact that this British Standard does not purport to include all the necessary provisions of a contract.

In order to keep abreast of progress in the industries concerned, British Standards are subject to periodical review. Suggestions for improvements will be recorded and in due course brought to the notice of the committees charged with the revision of the standards to which they refer.

A complete list of British Standards, numbering over 3000, indexed and cross-indexed for reference, together with an abstract of each standard, will be found in the Institution's Yearbook, price 15s.

This standard makes reference to the following British Standards:

B.S. 572. Interchangeable conical ground glass joints.
B.S. 658. Apparatus for the determination of distillation range.
B.S. 1282. Classification of wood preservatives and their methods of application.

British Standards are revised, when necessary, by the issue either of amendment slips or of revised editions. It is important that users of British Standards should ascertain that they are in possession of the latest amendments or editions.

The following B.S.I. references relate to the work on this standard:
Committee reference WPC/10 Draft for comment A(WPC)3716

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CO-OPERATING ORGANIZATIONS

The Wood Preservation Industry Standards Committee, under whose supervision this British Standard was prepared, consists of representatives from the following Government departments and scientific and industrial organizations:

*Association of British Chemical Manufacturers
*Association of Tar Distillers
*Board of Trade
*British Wood Preserving Association
*D.S.I.R.—Forest Products Research Laboratory
*D.S.I.R.—Laboratory of the Government Chemist
Electricity Council, the Generating Board and the Area Boards in England and Wales
English Joinery Manufacturers' Association (Incorporated)
Fencing Contractors' Association
Ministry of Agriculture, Fisheries and Food
Ministry of Aviation
Ministry of Power
National Coal Board
Post Office
*Timber Development Association
Timber Trade Federation of the United Kingdom

The Government departments and scientific and industrial organizations marked with an asterisk in the above list, together with the following, were directly represented on the Committee entrusted with the preparation of this British Standard:

Association of Clinical Pathologists
Biochemical Society
British Medical Association
Crown Agents for Oversea Governments and Administrations
Federation of Gelatine and Glue Manufacturers
Industrial Pest Control Association

BRITISH STANDARD METHODS OF TEST FOR

TOXICITY OF WOOD PRESERVATIVES TO FUNGI

FOREWORD

This British Standard was first published in 1939. In this revision the wood block method has been retained as the basic method of test for toxicity, but procedures are now given for determining both the initial toxicity and the residual toxicity after leaching and evaporation. A test for toxicity to soft-rot microfungi is also included.

The effectiveness of any substance for the preservation of wood depends on a number of factors* of which the most important are:

(i) Toxicity towards wood-destroying fungi, timber-boring insects and marine borers.

(ii) Degree of permanence, i.e. resistance to leaching, evaporation and chemical decomposition.

(iii) Penetrating power, e.g., low viscosity at temperatures at which the product is normally applied.

(iv) Freedom from undesirable effects on timber and metals, and from hazards to health.

In the present standard, only tests for initial toxicity to fungi and residual toxicity after exposure to leaching and evaporation are considered. It must be emphasized that, whilst laboratory testing provides a convenient and reasonably rapid method of evaluating these properties, it is not possible to estimate from the results of such laboratory tests alone the precise value in practice of any substance as a wood preservative, particularly if this is to be used on timber exposed out of doors to the influence of the weather, nor to forecast the probable life of timber structures treated in any particular way with the substance.

Further it must be emphasized that the amounts of preservative found necessary to inhibit fungal attack under the conditions specified in the test cannot be assumed to be those required for the protection of timber in service. This is because the methods of impregnation and quality of timber may not be comparable with those used in the laboratory tests, and because timber in use may be exposed to weathering influences which tend to lower the effective concentration of preservative in the wood and which may not be completely reproduced in laboratory leaching procedures.

In the past, various methods have been employed to determine the toxicity of wood preservatives, but in recent years two types of test, basically similar in

* For further information, see B.S. 1282, ' Classification of wood preservatives and their methods of application'.

principle, have generally been used. In Europe a wood block test similar to that described in the first edition of this standard has been generally adopted, whereas in America the so-called 'soil/block' method, in which soil and wood are used instead of nutrient agar, has been developed and recently adopted by the American Society for Testing Materials as a tentative standard.*

The relative values of these two methods have been discussed in several papers and the general conclusion seems to be that the results obtained by the two methods do not differ greatly. Slightly higher toxic limits for tar oils are generally obtained in the soil/block than in the agar/block method, as might be expected in view of the use of a smaller test block and a longer conditioning period in the former test. Control of the moisture content of the test blocks during the course of the test appears to be rather easier when soil is used. A disadvantage of the soil method is that handling of soil is more laborious and slower than that of agar solutions, and the test has been criticized on the grounds that soil is a variable material that cannot be standardized. Since the fungi in this method are, however, cultivated on a 'feeder' block of untreated wood resting on soil, this objection is probably of minor importance.

For the preliminary screening of chemicals as wood preservatives, determination of the minimum concentration inhibiting growth of the test fungi in malt agar (the so-called agar or Petri-dish test) may be of value for determining the toxicity.† It is most useful for determining the relative toxicity of substances of similar chemical character and of similar solubility in water, e.g., a series of phenols of different boiling points, but its use as a standard test for wood preservatives is not recommended.

Recent investigations have shown that decomposition of wood in contact with the soil and in water-cooling towers may be brought about by the action of certain cellulose-destroying microfungi which cause the so-called 'soft rot'. Preservatives intended for use in such situations should be tested according to the method laid down in Part 2, in addition to undergoing the tests laid down in Part 1 of the standard.

It is not intended that every test described in this British Standard should be carried out when information on the initial toxicity of a wood preservative is required; the appropriate tests should be selected according to the purpose for which the preservative is to be used.

This standard prescribes methods of test only and should not be used or quoted as a specification. A form of words indicating that a preservative conforms to B.S. 838 should not be used as it conveys no meaning.

NOTE. Where figures in British units are stated they are to be regarded as the standard, Any metric equivalents shown in parentheses are approximate only. More accurate conversions should be based on the tables in B.S. 350, ' Conversion factors and tables '

* A.S.T.M. Method D-56T.

† Cartwright and Findlay, ' Decay of timber and its prevention ', 2nd edn. (H.M.S.O., 1958). p. 292.

METHODS OF TEST

SCOPE

1. This British Standard describes methods of test for the initial toxicity of wood preservatives to fungi and the residual toxicity after exposure to leaching and evaporation. A test for toxicity to soft-rot microfungi is included.

The methods are applicable to tar-oil, water-borne and organic-solvent types of wood preservatives. They enable comparisons to be made between preservatives which all belong to any one of these types; great caution should be exercised in comparing preservatives of different types, since it is not possible to ensure that one test procedure will accurately take into account the different mechanisms by which various preservatives operate under service conditions.

PART 1. WOOD BLOCK METHOD (BASIDIOMYCETE TEST)

SECTION ONE: GENERAL

PRINCIPLE

2. The principle of this test is the exposure, to pure cultures of wood-rotting fungi growing under controlled conditions, of test pieces of a perishable type of wood that have received the prescribed treatment with the preservative under investigation. Control tests are carried out at the same time, using test pieces that have been treated with the solvent (if any is used in diluting the preservative solution) before exposure to the test fungi.

Wood preservatives are generally applied to timber in one of two ways, either (i) by impregnating the wood under pressure or (ii) by brushing or by steeping the wood in the fluid.* The basis of comparison between preservatives of the first type is the determination of the minimum quantity of preservative, expressed as kilogrammes per cubic metre (or pounds per cubic foot), that inhibits attack by the test fungi, and for type (ii) is whether or not fungal attack of the treated test pieces is prevented where the preservative has been applied according to the procedure described in Clause 9.

The susceptibility of the preservative to loss by leaching and evaporation is estimated by determining the residual toxicity of the preservative in test pieces that have received the arbitrary standard washing and heating treatment described in Clause 11*b* or Clause 11*c* and comparing this residual toxicity with the toxicity determined on samples that have not been so treated.

The leaching test described in Clause 11*b* should reveal whether the preservative under test has any tendency to leach from the wood after impregnation, but for preservatives for timber to be used under conditions of high leaching hazard, for example cooling towers, marine work and other timbers subject to persistent leaching, the more severe leaching test described in Clause 11*c* should be used.

* For further information, see B.S. 1282, ' Classification of wood preservatives and their methods of application '.

CULTURE VESSELS

3. Either flat culture flasks of the type known as Kolle flasks, or square glass bottles with wide mouths and screw tops may be used as culture vessels.

The dimensions of the Kolle flask shall be as shown in Fig. 1.

In the neck there is a reservoir for water formed by a second ridge (see Fig. 1) in which a pad of cotton wool soaked in water can be retained. To facilitate inoculation an inner tube is fitted through the large cotton wool plug closing the neck. This tube is closed by means of a small cotton wool plug.

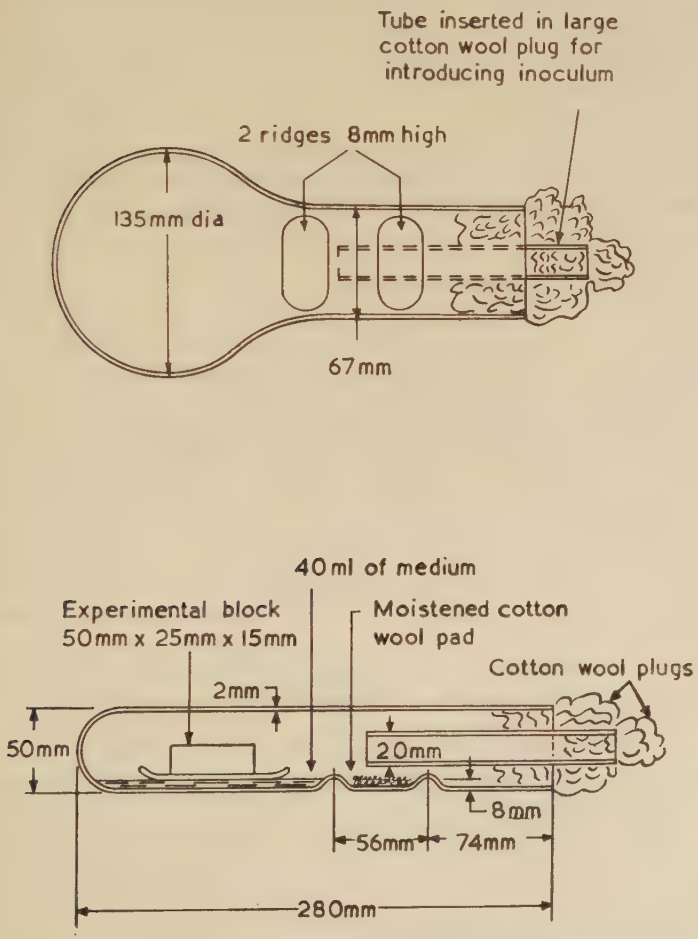


Fig. 1. Flask for wood block test of toxicity of wood preservative.

The bottles shall have a capacity of about 20 oz (approx. ½ litre). They shall be of square section about 70×70 mm externally and 170 mm long and shall have a neck of at least 45 mm internal diameter with an external thread to take a screw-on metal closure, preferably of aluminium. In the metal cap a round hole about 15 mm in diameter should be cut; this is plugged with cotton wool when in use.

MEDIUM FOR GROWING CULTURES

4. A nutrient medium shall be prepared by the following procedure. Heat, in an autoclave or steamer, 50 g of malt extract* or 40 g of dry powdered malt and 20 g of powdered agar in 1000 ml of distilled water†. Pour about 60 ml of this medium into each flask or bottle, then plug the vessel with cotton wool or close with the metal cap. Sterilize for 20 minutes at 15 lb/in² (1·1 kg/cm²) gauge pressure or by steaming at atmospheric pressure for 30 minutes on each of 3 successive days. After sterilization, lay the vessels flat so that the medium is retained by the ridge in the neck. The medium in the vessels should be inoculated with the test fungus within six days after its preparation.

TEST FUNGI

5. For tests on pine sapwood, at least three of the following species shall be used:

a. Coniophora cerebella Pers. (*Coniophora puteana* Fr.). This fungus should be used in all tests as it is resistant to many different preservatives.

b. Poria monticola Murr.

NOTE. Further examination of the Eberswalde strain of *Poria vaporaria* (referred to in the first edition of this British Standard) has shown that it is in fact a monosporous culture of *Poria monticola*.

c. Lentinus lepideus Fr. This fungus should be included in any tests with tar oils, to which it is particularly resistant.

d. Lenzites trabea (Pers.) Fr. This fungus is particularly resistant to arsenic and should therefore be used in tests of any preservatives that contain this element.

For tests on beech the following species shall be used:

Polystictus versicolor (Linn.) Fr. (*Coriolus versicolor* (Linn.) Quél.)

NOTE. These fungi have been chosen for the following reasons:

(i) they are resistant to antiseptics: e.g., *Poria monticola* is resistant to copper compounds.

(ii) they grow quickly and cause a rapid loss in weight of the test timbers.

(iii) they are not unduly sensitive to slight variations in environmental conditions.

* Malt extract of B.P. quality is suitable.

† When distilled water is mentioned in the text, water demineralized by ion-exchange resins may be used in its place.

(iv) they are species of economic importance.

(v) they include species chosen as test fungi in European and American standards.

Vigorous standard strains of these fungi, which may be obtained, for example, from the Department of Scientific and Industrial Research, Forest Products Research Laboratory, Princes Risborough, Bucks, shall be used. The source and strain number of the cultures used shall be recorded.

Subculturing of the test fungi is permitted but, if the appearance of the cultures should at any time change radically, fresh cultures should be obtained, to avoid the danger of subculturing a contaminating organism. With the exception of *Lenzites trabea*, none of these fungi produce aerial conidia, and careful microscopic examination of their hyphae should reveal some bearing ' clamp-connections '.

NOTE. As preservative testing involves maintenance of fungus cultures for long periods (up to 14 weeks or so), it is necessary to pay the strictest attention to general hygiene in the laboratory and in incubators used for storage. This is necessary to prevent infestation of the cultures by mites, which may introduce contaminating microfungi into the test flasks and may thereby invalidate the results of the tests.

The cultures shall be in active growth when the test blocks are introduced and in any case not more than one month old and free from contamination with other organisms.

The vessels containing the cultures and the test blocks shall be kept in an incubator at 22 $\pm$0·5°C, in which the relative humidity shall be not less than 65 per cent. If Kolle flasks are used and the medium shows signs of drying up, sterile water shall be added aseptically to moisten the cotton wool pad in the neck of the flask, in order to maintain conditions ideal for fungal growth in the flasks. If screw-top bottles are used it should not be necessary to add any water during the course of the test.

SPECIES OF WOOD

6. The species of wood used shall be:

a. the sapwood of Scots pine—*Pinus sylvestris.*

b. the outerwood (i.e., within about 3 in (8 cm) of the inside of the bark) of Beech—*Fagus sylvatica.*

NOTE. The above mentioned kinds of wood have been chosen because they are readily attacked by fungi (i.e., have little natural resistance to decay), are easily impregnated and readily procurable. Other perishable timbers and appropriate test fungi may be used in special investigations or where the species mentioned are not available.

The wood shall be clean, bright and dry, free from knots, normal in structure and of an average rate of growth (i.e., showing 8-20 annual rings to the inch). Pine or other coniferous softwood shall not be excessively or unusually resinous. Wood from the branches or from the base of the trunk near the roots shall not be used.

The test blocks shall be finished to a size of $5 \times 2{\cdot}5 \times 1{\cdot}5$ cm and cut so that the long axis is parallel to the grain of the wood.

The density of the Scots pine sapwood shall be such that the oven-dry weight of the blocks lies between 7·5 and 9·5 g and that of beech so that the weight of the blocks is between 10·0 and 12·0 g.

PREPARATION OF BLOCKS FOR TREATMENT

7. The blocks shall be dried and sterilized by heating in a drying oven at 100-105°C for about 18 hours. They shall then be weighed with aseptic precautions to the nearest centigramme: this weight is referred to as the 'initial dry weight'. In order to ensure sterility of beech blocks, it is necessary to autoclave them in steam at 15 lb/in² (1·1 kg/cm²) pressure for 20 min before oven-drying them.

SECTION TWO: TREATMENT OF WOOD BLOCKS WITH PRESERVATIVE

TREATMENT WITH PRESERVATIVES INTENDED FOR PRESSURE IMPREGNATION

8. A series of dilutions of the preservative, usually 6 in number, shall be prepared by dissolving weighed amounts of the substance under test in a suitable solvent. The concentrations, which shall be prepared on a weight/weight basis, shall be chosen so that approximately half of the series will fall above and half below the expected toxic limit (see Clause 15*a*). In the case of completely unknown substances it may be helpful to carry out a preliminary estimation of the toxicity by means of an agar plate test. In order to determine accurately the toxic limit, it is often necessary to carry out a second test using a more closely graded series of concentrations than in the first test. For the initial screening the concentrations should form a geometrical series but for the final test they should be in an arithmetical series.

If the preservative is soluble in water, freshly prepared aqueous solutions shall be used. Preservatives that are insoluble in water shall be dissolved in some volatile liquid which does not leave a residue that is toxic to fungi and whose distillation range when determined by means of the apparatus described in B.S. 658* is not lower than 60°C and preferably not higher than 90°C. Suitable liquids are light petroleum, acetone, benzene and ethanol. Viscous fluids of low toxicity, which need to be used at full strength, should be applied at a suitable elevated temperature.

The blocks shall be impregnated in the following manner.

Weight the blocks down in beakers (lead shot in sealed glass containers make convenient weights) and expose to a reduced pressure for ten minutes in

* B.S. 658, 'Apparatus for the determination of distillation range'.

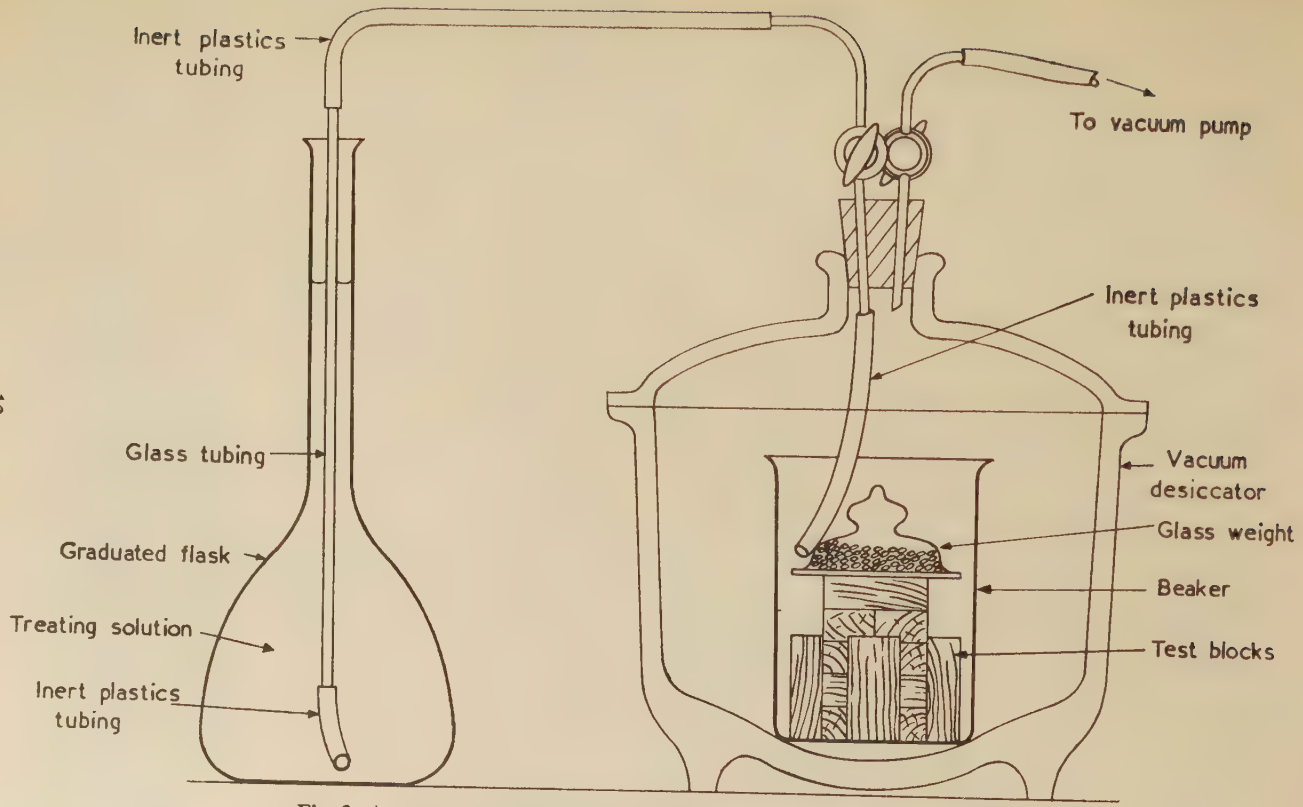


Fig. 2. Apparatus for impregnation of wood blocks with wood preservative.

a vacuum desiccator. Close the tube leading to the pump, and impregnate the blocks with the preservative solution, which is admitted through a tube leading to the beaker (see Fig. 2). An inert type of plastics material may be used to make flexible joints. Treatment with the solution of lowest concentration should be carried out first. When the blocks are well covered, release the vacuum, remove the beaker containing the blocks from the desiccator and leave it for two hours, by which time the blocks should be fully saturated. After the above treatment, stand the blocks on end on clean absorbent paper, allow to drain for not more than one minute and again weigh. Calculate the amount of preservative taken up by the blocks from the weight of liquid absorbed (= weight after treatment minus initial dry weight) and the concentration of the solution. It is usual to express the concentration of preservative as kilogrammes per cubic metre of wood (or as pounds per cubic foot).

Control blocks shall be treated in a similar manner by impregnation with the solvent employed for dilution of the preservative. It is desirable that from time to time an additional series of *untreated* control blocks should also be included in the test, to permit appraisal of the vigour of the test fungi and to confirm that there is no residual toxic effect from traces of solvent left after the drying procedure. Losses in weight of solvent-treated and untreated control blocks should not be significantly different.

Uniform penetration of the blocks is necessary in order to obtain satisfactory replication of the test results, but strict control is difficult. The treatment of pine test blocks shall not be considered satisfactory unless the blocks have absorbed more than 100 per cent of their oven-dry weight of any preservative solution having a specific gravity of 1·0 or over, or 75 per cent of any solution with a specific gravity significantly less than 1·0. For beech blocks no general figures can be quoted, but the following examples are given as a guide to the *minimum* absorptions which should be obtained with solutions in various solvents.

Solvent	Minimum absorption (per cent of oven-dry weight)
Water	80
Acetone	60
Ethanol	60
Benzene	50
Light petroleum	40

If in any one group of samples treated at the same time the weight of solution absorbed by any particular block differs by more than 15 per cent from the average absorption of the batch, the results from such blocks shall not be included in the average for the group. Not less than four blocks per fungus shall be treated at each concentration and the results from these four samples shall be averaged, provided that the absorptions do not differ by more than the

figure stated above. If variable absorptions are being obtained, extra blocks should be included to replace those rejected. Four control blocks treated with solvent only shall be prepared for exposure to each of the test fungi. If the preservative is partly volatile at 100°C, four extra blocks of pine and beech at each concentration shall be included to determine the proportion of absorbed preservative lost during the various operations (see Clause 14).

The solvent used shall be stated in the test report.

TREATMENT WITH PRESERVATIVES INTENDED FOR SURFACE APPLICATION

9.*a. Introduction.* Although the initial toxicity of preservatives intended for application by the surface treatment methods of brushing, dipping or spraying can be determined after using the impregnation treatment described in Clause 8, it would be unrealistic to base assessment of such preservatives on a test in which the method of application bears no relation to that employed in practice. It is therefore preferable that treatment of test blocks with preservatives intended solely for surface application should be carried out by surface application of the preservatives at the concentrations recommended for use by their manufacturers. The precise control of application made possible by the impregnation method of treatment cannot be achieved, and modifications in the system of reporting test results are required so that the results themselves will bear a relation to the mode of application as well as to the initial toxicity of the chemicals employed.

The test described is arbitrary, and subject to greater variability than that based on vacuum impregnation; nevertheless it is of practical value when the results are interpreted in the light of experience.

b. Treatment. The oven-dried and weighed blocks shall be immersed for periods of (i) 10 seconds, (ii) 10 minutes, in the preservative and then allowed to drain for one minute on clean absorbent paper before being re-weighed to determine the uptake of preservative per block; these absorptions shall be reported. The absorption of preservative shall not in this case be expressed as kilogrammes per cubic metre since the blocks are not treated throughout (see Clause 15 *b*).

NOTE. The periods of immersion specified are believed to give retentions corresponding approximately to those achieved by one and three brush applications, respectively.

Six blocks treated at each of the periods, and four untreated but sterilized blocks shall be exposed to each of the test fungi.

SECTION THREE: CONDITIONING OF BLOCKS AFTER TREATMENT

CONDITIONING FOR DETERMINATION OF INITIAL TOXICITY

10. Blocks that have been impregnated with an aqueous solution shall be dried down until about two thirds of the absorbed water has evaporated. Blocks

that have been impregnated with an organic solvent shall be dried out until all the solvent has evaporated. The following procedure should achieve the necessary degree of drying, but drying ovens vary in efficiency, particularly if they are fully loaded.

After treatment, stand the blocks on end and leave them for 72 hours freely exposed to the air of a normally heated laboratory. Then dry the blocks for a further 3 hours at 50±1°C in a ventilated oven provided with a circulating fan. Immediately after removal from the oven, stand the blocks on end in a desiccator into which sufficient 1:2-epoxypropane has been introduced to saturate the atmosphere. Leave the blocks overnight exposed to the vapour of this compound.

Before taking the blocks out of the desiccator, remove this vapour by passing filtered air through the container for 10 minutes. Using a pair of sterilized forceps, then place the blocks directly in the culture flasks.

CONDITIONING FOR DETERMINATION OF RESIDUAL TOXICITY AFTER EXPOSURE TO LEACHING AND EVAPORATION

11. *a. General.* A duplicate series of test blocks shall be impregnated with preservative as described above. If the preservative is of the water-borne type in which fixation of the salts requires some time for its completion, the treated blocks shall, after impregnation, be kept for two weeks in a closed vessel out of contact with one another, exposed to the vapour from about 5 ml of toluene to prevent growth of moulds, and for a further two weeks freely exposed to the air of the laboratory before receiving the treatment described below.

Blocks that have been impregnated with a preservative dissolved in an organic solvent shall be allowed to dry for four weeks freely exposed in the laboratory.

b. Leaching procedure (*beaker method*). The dry test blocks shall be fully impregnated with distilled water by means of a vacuum filter pump as described in Clause 8.

The test blocks shall then be placed in glass vessels and covered with distilled water. Only blocks that have been treated with the same concentration of the same preservative shall be placed together in one container. The volume of water in each container shall be three times the total volume of all the test blocks in that container. For example, 8 test blocks each 5×2·5×1·5 cm in a beaker of 800 ml capacity shall be covered with 450 ml of water.

The vessels containing the test blocks may be covered with a piece of netting secured with a rubber band, to retain the test pieces while the water is changed.

The water covering the test blocks shall be changed three times in every 24 hours, for example at 9 a.m., 1 p.m., and 5 p.m. Care shall be taken to change the arrangement of the blocks.

After four days' exposure to washing at laboratory temperature the test blocks shall be removed from the containers and placed in a well ventilated

drying oven (provided with a fan) maintained at 50°C for a period of three days (72 hours), after which treatment they shall again be impregnated with distilled water in the same manner as before. This leaching and drying treatment shall be repeated three times so that the blocks receive, in all, sixteen days' washing and twelve days' drying. The blocks shall then be sterilized as described in Clause 10.

c. Alternative leaching procedure (*Soxhlet method*). If it is required to test the resistance of the preservative to severe leaching, the following treatment shall be used instead of the repeated washing and drying treatment described in Clause 11*b*.

The test blocks after impregnation and pre-conditioning as described in Clause 10 shall be continuously extracted with condensed water at 40±5°C in a Soxhlet-type apparatus, as specified in Appendix B and shown in Fig. 4. The extraction chamber in this apparatus will hold 16 test blocks of size 5×2·5×1·5 cm or 60 blocks of size 5×2·5×0·5 cm (see Clause 20).

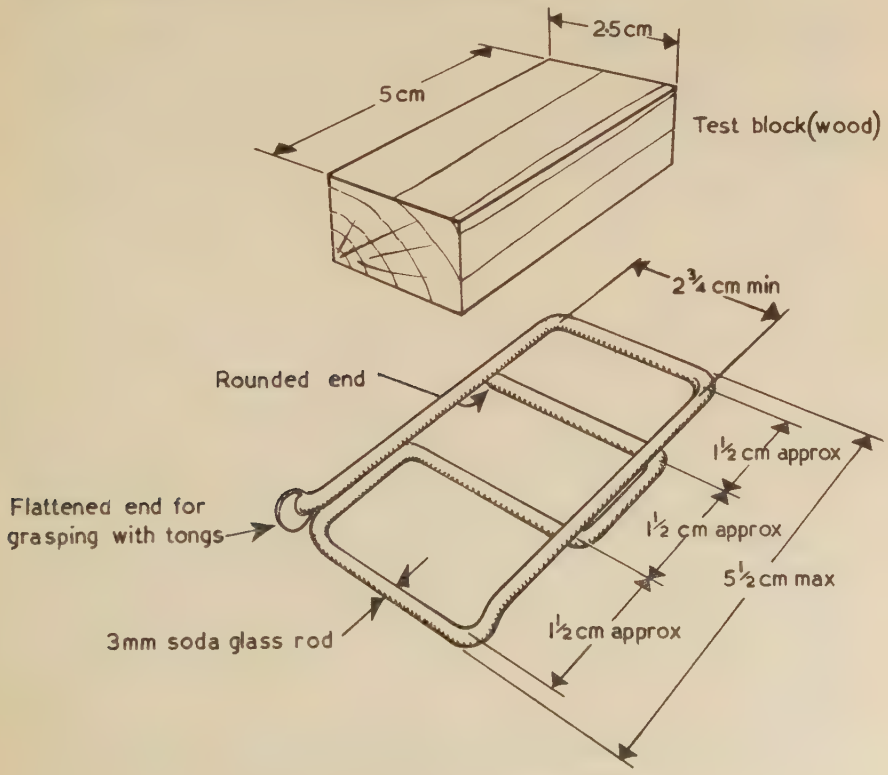


Fig. 3. Glass frame for supporting test block.

To carry out the treatment, put at least 500 ml of distilled water into the boiling vessel and insert the test blocks into the extraction chamber. Assemble the apparatus, switch on the heater and adjust the rate of heating to give a leaching cycle of approximately 60 minutes, the rate of steam condensation in the condenser being such that the condensed water has a temperature of $40 \pm 5°C$.

For blocks 1·5 cm thick, as specified in Clause 6, the total leaching period shall be 300 hours. For blocks 0·5 cm thick, as specified in Clause 20, the total leaching period shall be 100 hours.

NOTE. With certain types of preservatives it may be found necessary to change the water in the flask after half the extraction period has elapsed.

SECTION FOUR: TEST PROCEDURE

INFECTION OF BLOCKS

12. The blocks shall be exposed to fungal attack by placing them aseptically in flasks or bottles in which there are actively growing cultures of the test fungi. Not less than four blocks treated with each concentration shall be exposed to each of the test fungi. Not more than two treated blocks shall be placed in each vessel; these shall have been treated with the same concentration of preservative.

The blocks should be placed on small glass frames as shown in Fig. 3, which allow the blocks to come into contact with the aerial mycelium of the fungus but not with the medium itself, into which some of the preservative might otherwise leach.

DURATION OF TEST

13. The test blocks shall remain exposed to fungus for three months.

EXAMINATION OF TEST BLOCKS AFTER EXPOSURE TO ATTACK

14. After exposure to fungal attack the blocks shall be removed from the flasks and examined for evidence of decay. Any blocks in which the growth of the test fungi has obviously been checked by contaminating organisms shall be rejected. The blocks shall then be freed from any adhering fungal mycelium and examined for any visible alteration. They may be tested for incipient decay by gently indenting them with a finger nail or the tip of a knife, care being taken not to lose any of the wood substance. They shall then be weighed, oven-dried at 100-105°C for eighteen hours and weighed again. The blocks shall again be examined for evidence of decay, which may have become more obvious on drying.

The latter weight shall be designated the ' final dry weight '. From these two weights the moisture content may be determined in order to check whether conditions have been suitable for fungal decay. The test blocks should neither

be waterlogged nor contain less than the average fibre-saturation value for the timber concerned.* The amount of decay that has occurred shall be estimated by the loss in dry weight brought about by the fungus. This loss shall be obtained by subtracting the final dry weight from the initial dry weight; an addition shall be made to the initial dry weight, to allow for the dry weight of preservative absorbed, only when the concentration of the treating solution exceeds 1 per cent of a salt or 2 per cent of an oil. When the preservative is partly volatile at 100°C, a complication arises because part of the absorbed preservative is evaporated, this giving an apparent loss of wood substance. Allowance can be made for this loss in weight, due to partial evaporation of preservative, by having a series of blocks treated in exactly the same way as those exposed to fungal attack, except that the flasks in which they are kept contain no fungal cultures (see Clause 8). The losses in weight due to evaporation of preservative determined for these blocks can then be applied as a correction to the experimental series and the true losses due to decay thus determined.

The loss in dry weight resulting from decay shall be expressed as a percentage of the initial dry weight of the wood. A loss in dry weight of less than 3·0 per cent shall be considered as falling within the limits of experimental error and shall be ignored for the purpose of determining the toxic limit.

The observations made by physical examination, being intended to show whether rotting of the test pieces has occurred, shall be reported and shall be used to check that the loss in weight is due to the test organism and not to other causes.

EVALUATION OF RESULTS

15. *a.* The basis on which the toxicity of the wood preservative to fungi shall be expressed quantitatively is the minimum quantity of the preservative in the wood which inhibits decay by the test fungi. This quantity shall be given as kilogrammes of preservative per cubic metre of wood (or pounds per cubic foot). The concentration of the treating solution found necessary to give this figure and the species of wood shall also be stated. The *toxic limit* is given as the interval between that concentration which just permits decay and the concentration next highest in the series which inhibits all decay.

If tests have been carried out to determine the resistance of the preservative to leaching and evaporation, the toxic limits of the residual preservative should be set out in the report alongside those determined for the same preservative obtained before exposure to leaching and evaporation.

b. The effectiveness of preservatives applied superficially by dipping for 10 seconds and 10 minutes cannot be given as a toxic limit, but the results should be reported as the number of blocks that have resisted decay and remained sound out of the total number exposed to each of the test fungi.

* The fibre-saturation value is usually about 27 per cent, calculated on the final dry weight.

PART 2. METHOD OF TEST FOR TOXICITY TO SOFT-ROT FUNGI

INTRODUCTION

16. As explained in the Foreword, it is desirable to test, against certain micro-fungi that cause soft rot, any preservative that is to be employed for the preservation of timber in contact with the soil or with water in water-cooling towers. Since timber in these situations is always exposed to severe leaching, the test described below should always be carried out in duplicate, one set of samples being tested without leaching and one set after the leaching treatment described in Clause 11*b* or 11*c*, as appropriate (see Clause 2), and the results compared.

While soft rot is known to be due to a number of organisms and occurs in softwoods as well as in hardwoods, experience of laboratory test procedures has been gained only for one fungus, *Chaetomium globosum*, and as far as is known the methods described below are applicable in detail only to this fungus on non-durable hardwoods. It is recognized that the range of test organisms should be extended when suitable experience has been gained, though possibly the use of other fungi may involve modification of details of the test procedure.

The method of test described below is based on a method designed for testing rot-proofed textiles and may be described as the ' mycelial mat method '.

CULTURE VESSELS

17. Square bottles of ' 20 oz ' capacity (approx. ½ litre), with screw-type metal lids, preferably of aluminium (without holes) shall be used.

MEDIUM

18. The fungi that cause soft rot in wood bring about significant losses in weight under the conditions of test only if certain additional nutrient salts are available to them, and the following medium shall be used:

Ammonium nitrate, NH_4NO_3	3·0 g
*di*Potassium hydrogen orthophosphate K_2HPO_4	2·0 g
Potassium dihydrogen orthophosphate KH_2PO_4	2·5 g
Magnesium sulphate $MgSO_47H_2O$	2·0 g
Agar	20 g
Distilled water	1000 ml.

75 ml of this medium which has been heated and melted in a steamer or autoclave is poured into each bottle. The bottles containing the medium are then sterilized for twenty minutes at 15 lb/in² (1·1 kg/cm²) gauge pressure in an autoclave and on removal are laid on one of the flat sides and allowed to cool.

INOCULATION

19. Test-tube cultures of *Chaetomium globosum* Kunze on malt agar should be started about three weeks before the test is undertaken, if the cultures are grown at room temperature.

A suspension of spores shall be prepared; this may be done conveniently by adding a few drops of sterile distilled water to the test tubes containing the culture and then scraping the surface of the fungus with a stiff sterilized wire bent at a right angle near the tip, to liberate the spores. Sufficient distilled water is then added to cover the slope and the tube is shaken vigorously to break up the spore masses. This spore suspension may then be filtered through sterile glass wool in a sterile funnel to remove the larger particles that might block the orifice of the sprayer. An atomizer that can give a fine spray and which can be detached from and sterilized separately from its rubber tubing and bulb, should be used. An air filter consisting of a piece of wide glass tubing packed loosely with sterile cotton wool should be introduced between the rubber bulb and the atomizer.

Two sterile pieces of filter paper* 5×5 cm shall be placed on the surface of the medium in each bottle and sprayed with the suspension of spores. The bottles shall then be incubated for three days at $30 \pm 0{\cdot}5°C$, by which time the growth should have become visible. If left to grow for a longer time fructifications may be formed which prevent good contact between the blocks and the medium.

SPECIES OF WOOD

20. The wood used shall be the outer wood of beech (*Fagus sylvatica*) as specified in Clause 6. The test pieces shall be finished to $5 \times 2{\cdot}5 \times 0{\cdot}5$ cm and cut so that the long axis is parallel to the grain of the wood.

TREATMENT OF BLOCKS

21. Not less than ten blocks shall be treated, as described in Part 1, with each concentration of the preservative.

INFECTION OF BLOCKS

22. Six blocks treated at each concentration of preservative and six untreated control blocks shall be exposed to attack by the fungus. They shall be exposed by being placed firmly on the three days old mycelial mat, not more than two blocks being placed in each bottle, in such a way that they do not touch one another, the two blocks in any one bottle having been treated with the same concentration of preservative. The lids shall not be screwed on so tightly that interchange of gases with the outside atmosphere is prevented.

Four blocks treated at each concentration of preservative and four untreated controls shall be placed as described above, on filter paper on agar in bottles

* Whatman No. 1 or Barcham Green No. 401 is suitable.

prepared as described in Clauses 18 and 19 but not inoculated with fungus. About 0·5 ml of toluene may be placed in each bottle to assist in maintaining sterile conditions, and the bottles may be tightly sealed with lids into which capseals or washers have been inserted.

DURATION OF TEST

23. The test blocks shall remain exposed to fungal attack for six weeks at $30 \pm 0{\cdot}5°C$, under conditions which are not conducive to rapid drying out (see Clause 5).

EXAMINATION OF TEST BLOCKS

24. The examination of the test blocks and the evaluation of the results shall follow the lines laid down in Clauses 14 and 15, with the following addition:

A correction shall be made to the loss in weight of the test blocks to allow for weight changes caused by contact with the nutrient agar medium. The correction factor employed shall be the average change in weight of the four test blocks treated with each concentration of preservative and incubated under sterile conditions.

A quick indication of the presence of soft rot in the test pieces can usually be obtained by fixing the blocks, after they have been dried and weighed, in a vice and striking them smartly with a light hammer on the side that was in contact with the medium. Rotted blocks break off with a clean brash fracture without splintering. It may be also noted that the amount of superficial growth of *Chaetomium globosum* on the blocks is usually a good indication of the amount of decay that has occurred.

APPENDIX A

DESCRIPTION OF TEST FUNGI

Coniophora cerebella. This fungus forms a soft mat which tends to form fine branching strands. It is pale yellow at first, later dark brown patches develop.

Lentinus lepideus. This fungus grows rather more slowly than the other test fungi. It forms at first a thin colourless mat, later forming a tufted woolly mat, which in the light shows patches of a pale brown or purplish brown. In old cultures cylindrical outgrowths about 0·5 cm thick and several centimetres long often develop at the upper end of the slope. This fungus always has a definite aromatic odour.

Poria monticola. This fungus forms an even white soft cotton-woolly mat sometimes with small fragile fruit bodies.

Polystictus versicolor. This fungus forms at first a thin mat of colourless mycelium appressed to the medium and later forms a thin white skin, which is fairly tough. It always tends to bleach the malt agar on which it is growing.

Lenzites trabea. This fungus forms at first a soft whitish fluffy cushion which quickly assumes a yellowish buff coloration. Later the centre of the mat may become closely appressed to the medium and sodden following the development of numerous secondary spores. Growth is somewhat more rapid than that of *Lentinus lepideus.*

Chaetomium globosum. Cultures of this fungus are at first whitish, but after a few days fruiting bodies develop profusely and eventually form an uneven, hairy, olive-green mat over the surface of the medium. The medium turns a brownish colour, and at the top of slope cultures the bases of fruit bodies can be seen through the medium as small dark dots. In the test bottles after three days' incubation at 30°C, there forms a thinly tufted colourless growth projecting a few millimetres from the filter paper. Growth is extremely sparse away from the filter-paper. After further incubation fruit body initials can be seen. At first these are very small and colourless but later increase in size to form small greenish or more often brown hairy ovoid bodies scarcely more than 2 mm high which may cluster thickly together on the wood but always occur very sparsely on the plain agar.

APPENDIX B

SOXHLET-TYPE LEACHING APPARATUS

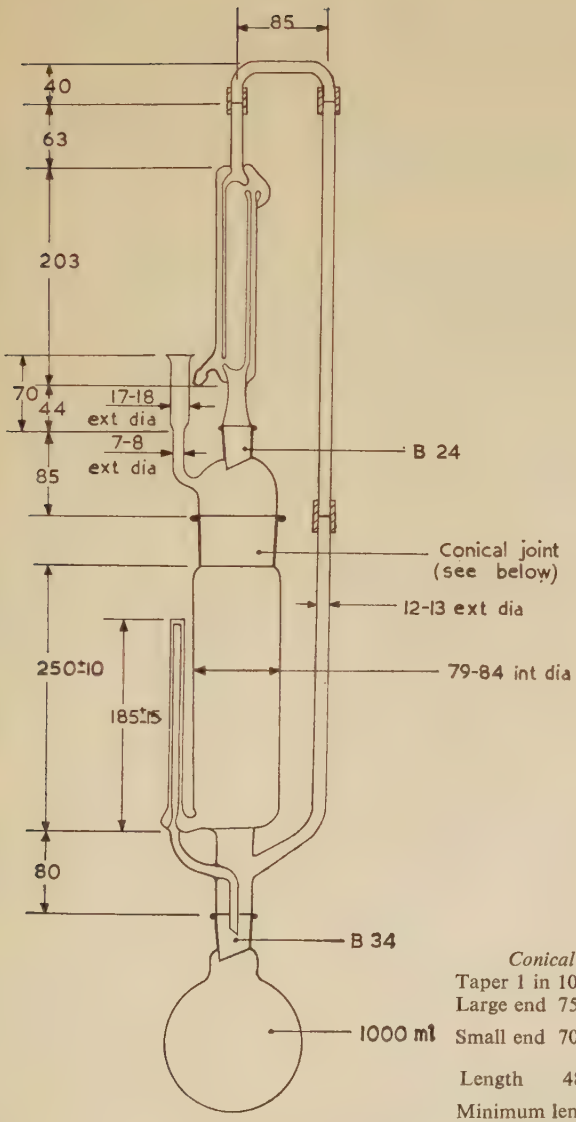
This apparatus is based on that described by Richardson and Larner* and is shown in Fig. 4. Details of the apparatus are as follows:

a. The double-surface condenser unit is fitted with a B 24 cone† and a converter unit, to which is fitted a water make-up arm, connects the condenser unit to the extraction chamber.

b. The height of the extraction chamber is 250 ± 10 mm and the internal diameter is 79-84 mm. The height of the siphon unit is between 170 and 200 mm from the base of the extraction chamber. The delivery tube at the base of the extraction chamber is fitted with a B 34 cone†, which is inserted into a B 34 socket† fitted to a 1000 ml boiling flask.

c. The boiling flask rests on a thermostatically controlled electric heating unit consisting of a spiral 1 kW heating element. A multiple unit comprising a number of individually controlled heaters may be used for tests run in parallel.

* Richardson, N. A. and Larner, E. E., *J. Soc. Chem. Ind.*, 1939, 58, 66.
† B.S. 572 ' Interchangeable conical ground glass joints '.



Conical joint above extractor

Taper 1 in 10

Large end $75 \pm 0{\cdot}1$ mm dia.

Small end $70{\cdot}2 \begin{smallmatrix} + 0{\cdot}1 \\ - 0{\cdot}4 \end{smallmatrix}$ mm dia.

Length $48 \begin{smallmatrix} +4 \\ -0 \end{smallmatrix}$ mm

Minimum length of engagement 46 mm

Fig. 4. Soxhlet-type leaching apparatus.
All dimensions in millimetres.

APPENDIX C

EXAMPLES OF TEST RESULTS

TABLE 1. EXAMPLE OF EVALUATION OF WOOD BLOCK (BASIDIOMYCETE) TEST

Substance: Commercial sodium fluoride.
Solvent: water.
Test fungus: *Coniophora cerebella*, F.P.R.L. No. 11A.
Timber: Scots Pine sapwood.
Period of test: 3 months at 22°C (without leaching).

Concentration of testing solution	Initial dry weight	Weight After treatment	Preservative in wood	Final wet weight	Final dry weight	Moisture content	Loss in dry weight	Loss in dry weight	Observations
per cent	g	g	kg/m³	g	g	per cent	g	per cent	
0·08	8·24	20·1		14·8	8·20	80·5	0·04	0·48	Sound
	7·95	19·8	0·51	11·5	7·87	46·1	0·08	1·01	
	8·37	20·3		13·0	8·30	56·6	0·07	0·83	
	8·54	20·5		15·6	8·45	84·6	0·09	1·05	
	Average loss in dry weight *0·84* per cent								
0·06	8·60	20·8		14·2	8·49	67·2	0·11	1·28	Sound, slight superficial growth of fungus
	8·75	20·9	0·38	12·0	8·64	38·9	0·11	1·26	
	8·59	20·7		13·6	8·51	59·8	0·08	0·93	
	8·49	20·1		14·0	8·37	67·3	0·12	1·41	
	Average loss in dry weight = *1·22* per cent								
0·04	8·45	19·9		12·6	8·16	54·4	0·29	3·43	Slightly decayed
	7·85	19·8	0·25	13·1	7·50	74·7	0·35	4·46	
	7·79	19·6		12·9	7·40	74·3	0·39	5·00	
	8·11	19·6		13·4	7·63	75·6	0·48	5·92	
	Average loss in dry weight = *4·7* per cent								
0·02	8·36	21·0		8·1	5·07	59·8	3·29	39·3	Three severely decayed
	8·27	20·9	0·13	22·0	8·12	170·0*	0·15*	1·81*	
	8·30	21·0		7·5	4·86	54·4	3·44	41·4	
	8·46	20·8		8·1	4·99	62·3	3·47	41·0	
	Average loss in dry weight = *40·54* per cent (average of 3 blocks)								
0 Controls	7·83			7·8	4·01	94·5	3·82	48·8	Severely decayed
	7·76			7·4	3·86	91·7	3·90	50·3	
	8·00			7·4	3·95	87·3	4·05	50·6	
	8·47			7·6	4·36	74·3	4·11	48·5	
	Average loss in dry weight = *49·5* per cent								

Toxic limit: 0·25–0·38 kg/m³.

* Result rejected because sample too wet.

TABLE 2. EXAMPLE OF EVALUATION OF RESULTS OF SOFT ROT TEST (BLOCKS LEACHED BY BEAKER METHOD)

Substance: Copper naphthenate, 8 per cent copper.
Solvent: Light petroleum, b.p. 60–80°C.
Test Fungus: *Chaetomium globosum*, F.P.R.L. No. S. 70B
Timber: Beech.
Period of test: Six weeks at 30°C.

Concentration of Testing solution	Initial dry weight	Weight after treatment	Absorption	Preservative in wood	Final dry weight	Loss or gain in weight	Corrected loss in dry weight	Corrected loss in dry weight	Conditions of sample at end of test	Type of fracture
per cent	g	g	g	kg/m³	g	g	g	per cent		
7·5	3·62	5·5	1·88		3·67	+0·05	0·05	1·38		
	3·97	5·8	1·83		3·91	−0·06	0·16	4·03		
	3·87	5·7	1·83		3·92	+0·05	0·05	1·29	Sound	Normal
	3·61	5·5	1·89		3·64	+0·03	0·07	1·94		
	3·69	5·6	1·91		3·74	+0·05	0·05	1·36		
	3·49	5·3	1·81	22·5	3·55	+0·06	0·04	1·15		
	3·78	5·8	2·02		3·90	+0·12	Average gain			
	3·65	5·5	1·85	Not exposed	3·72	+0·07	in weight			
	3·82	5·7	1·88	to fungus	3·92	+0·10	(Correction)			
	4·07	5·9	1·83		4·18	+0·11	= 0·1 g			
	After correction the average loss in dry weight = 1·9 per cent									
5	3·79	5·5	1·71		3·47	−0·32	0·37	9·76	Decayed	Brash
	3·81	5·8	1·99*		3·83*	+0·02*	0·03*	0·79*	Sound	Normal
	3·70	5·3	1·60		3·54	−0·16	0·21	5·68	Decayed	Brash
	4·17	5·8	1·63		4·08	−0·09	0·14	3·36	Partly decayed underside	Brash
	3·68	5·2	1·52		3·70	+0·02	0·03	0·82	Sound	Normal
	3·77	5·4	1·63	13·3	3·80	−0·03	0·02	0·55	Sound	Normal
	3·82	5·4	1·58		3·89	+0·07	Average gain			
	3·78	5·6	1·62	Not exposed	4·07	+0·09	in weight			
	3·86	5·6	1·74	to fungus	3·87	+0·01	(Correction)			
	3·95	5·6	1·65		3·91	+0·04	= 0·05 g			
	After correction the average loss in dry weight = 4·0 per cent									
2·5	3·82	5·7	1·88		3·30	−0·52	−0·56	14·7		
	3·82	5·2	1·38†		3·21†	−0·61†	−0·65†	17·0†		
	4·07	5·8	1·73		3·28	−0·79	−0·83	20·4	Decayed	Brash
	3·68	5·4	1·72		2·85	−0·83	−0·87	23·6		
	3·82	5·4	1·58		2·88	−0·94	−0·98	25·7		
	3·81	5·3	1·49	6·62	3·28	−0·53	−0·57	15·0		
	3·89	5·7	1·81		3·90	+0·01	Average gain			
	4·02	5·9	1·88	Not exposed	4·06	+0·04	in weight			
	3·85	5·3	1·45	to fungus	3·87	+0·02	(Correction)			
	3·78	5·4	1·62		3·87	+0·09	= 0·04 g			
	After correction the average loss in dry weight = 19·9 per cent									

The toxic limit after leaching is 13·3–22·5 kg/m³.

* Results rejected because absorption is outside permissible range (1·42–1·92 g).

† Results rejected because absorption is outside permissible range (1·40–1·90 g).

APPENDIX D

EXAMPLE OF EFFECT OF LEACHING ON A PRESERVATIVE

Fungus	Timber	Toxic limits of fully impregnated test blocks			
		Unleached series		Leached series (beaker method)	
		Concentration of treating solution	Weight of preservative in wood	Concentration of treating solution	Weight of preservative in wood
		per cent	kg/m³	per cent	kg/m³
Coniophora cerebella	Scots pine sapwood	0·25–0·5	1·8–3·7	0·5–1	3·6–7·3
Poria monticola	Scots pine sapwood	0·075–0·1	0·5–0·7	0·25–0·5	1·7–3·6
Polystictus versicolor	Beech	0·1–0·25	0·6–1·6	2–3	13–20

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